REMARKS

Applicant intends this response to be a complete response to the Examiner's 3 November 2006 Final Office Action. Applicant has labeled the paragraphs in his response to correspond to the paragraph labeling in the Office Action for the convenience of the Examiner.

EXECUTIVE SUMMARY

1. **Korlach et al. did not disclose beta or gamma labeled nucleotides until 17 May 2000 – the Korlach et al. 1999 provisional *did not* disclose beta or gamma labeled nucleotides.**

2. **Applicants invented their technology before 17 May 2000 and submit a Declaration and excerpts from a document to support prior invention.**

3. **The claims of the *cited* Korlach et al. application are not supported by the 17 May 2000 Korlach et al. filing and cannot be relied upon as a basis for rejecting the claims of Applicants' application – *the new Korlach et al. claims were copied from Applicants' application.***

4. **Korlach et al. did not disclose using beta or gamma labeled nucleotides and labeled polymerase in a tag interactive format such as FRET.**

5. **Schneider et al. did not disclose beta or gamma labeled nucleotides at all, even though they focused their application on interactive tag technology.**

Thus, neither Korlach et al. nor Schneider et al. taught or invented using beta or gamma labeled nucleotides and labeled polymerase in a tag interactive format such as FRET - a strong teaching away from the claims of Applicants' application.

DETAILED ACTION

Applicant's amendments and remarks, filed 6/7/06 and 8/22/06, are acknowledged. Amended claims 10, 13, 14, and 15 and new claims 57-99 are acknowledged.

The Examiner contends as follows:

> Applicant's arguments, filed 6/7/06, have been fully considered but they are not deemed to be persuasive. Rejections and/or objections not reiterated from the previous office actions are hereby withdrawn. The following rejections and/or objections are either reiterated or newly applied. They constitute the complete set presently being applied to the instant application.
>
> Claims 10, 13-19, and 50-99 are herein under examination.

Applicants acknowledge these statements. Applicants have documentary support for an earlier invention date, but the delay in raising these issues has complicated the manner of disclosing this information to the Patent Office, without prejudicing Applicants in the event of an upcoming

interference. However, Applicants are submitting a Rule 131 declaration with documentary support (with actual dates redacted) to evidence prior invention under the penalties of 18 U.S.C. § 1001. Applicants expect this declaration to remove Korlach et al. as a 102 reference against the present invention, causing those claims to become allowable and if necessary, requiring the invocation of an interference for other claims. However, Applicants believe that all of the claims of this application are patentably distinct over Korlach et al. or a combination of Korlach et al. and Schneider et al., especially when the two teaching are simultaneously considered.

Claim Objections

Claim 79 stands objected to because of the following informality:

The Examiner contends as follows:

> Claim 79 recites "a site" in singular form in line 2, but later states "are not sites" in plural form on line 8. Appropriate correction is required.

Applicants have amended claims 79 to address this informality and respectfully request withdrawal of this objection.

Claims Rejected Under 35 USC § 112, first paragraph

Claims 10, 13-19 and 79-99 stand rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor, at the time of the invention was filed, had possession of the claimed invention.

The Examiner contends as follows:

> Applicants point to support for the amended claim limitations on pages 20-21, 40-41, 45-52, 58, and 72-73. There does not appear to be adequate written support for the following limitations: "and where the polymerizing agent lacks 3' to 5' exonuclease activity" (claim 10) and "lacking 3' to 5' exonuclease activity" (claims 13 (pertaining to polymerase on line 2), 14 (pertaining to T7 DNA polymerase on line 3 and pertaining to the Klenow fragment from E. coli DNA polymerase I on line 4)), and the polymerizing agent "lacking 3' to 5' exonuclease activity" (claim 79, line 2). While there is written support for Taq DNA polymerase I lacking 3' to 5' exonuclease activity (page 4, first full paragraph and page 40, fourth paragraph) and Taq DNA polymerase I, Sequenase, and reverse transcriptase including HIV-1 reverse transcriptase inherently lack 3' to 5' exonuclease activity (see Wisniewski et al. (Journal of Biological Chemistry, 1999, Volume 274, Number 40, pages 28175-28184) and Gardner et al. (Nucleic Acids Research, 2002, Volume 30, Number 2, pages 605-613)), there is no written support regarding this lack of 3' to 5' exonuclease activity nor is this activity inherent for T7 DNA polymerase and the

Klenow fragment from E. coli DNA polymerase I (see USB Molecular Biology Reagents/Protocols 1992, pages 150 and 152) in the originally filed application, as now stated in amended claims 13 and 14. It is noted that page 72 (fourth paragraph) of the specification states T7 DNA polymerase has exonuclease activity. In addition, there does not appear to be written support for a polymerizing agent and polymerase lacking 3' to 5' exonuclease activity (as stated in instant claims 10, 13, and 79) which encompasses the above-mentioned non-inherent polymerases lacking 3' to 5' exonuclease activity that do not have written support.

There does not appear to be adequate written support for the phrase "where the site comprises a naturally occurring cysteine site or a cysteine replacement site in the polymerizing agent selected so that the site is less than or equal to about 25Å from a tag on each incorporating monomer regions and are not sites having structural/functional importance to proper functioning of the polymerizing agent and is covalently bonded to the cysteine through its SH group" (claim 79) and "the site comprises a naturally occurring cysteine site or a cysteine replacement site in the polymerizing agent selected so that the site is less than or equal to about 25A from a tag on each incorporating monomer regions and is covalently bonded to the cysteine through its SH group" (claim 89). There does not appear to be adequate written support for "the site is less than or equal to about 15Å from a tag on each incorporating monomer" (claims 80 and 90) and "the site is less than or equal to about 10A from a tag on each incorporating monomer" (claims 81 and 91). Written basis is provided for a cysteine replacement site (page 46, third paragraph), but not "naturally occurring cysteine site" which differs in scope. While there is written basis for distance separating tags to be within about 25Å, 15Å, and 10Å (page 18, last paragraph and page 20, second paragraph), there does not appear to be written support for "the *site* is less than or equal to about 25Å from a tag on each incorporating monomer regions", "the *site* is less than or equal to about 15Å from a tag on each incorporating monomer", and "the *site* is less than or equal to about 10Å from a tag on each incorporating monomer" which differ in scope. Written basis is provided for cysteine replacement preferred sites that are not in contact with other proteins, do not alter conformation of the polymerase, and are not involved in protein function (page 46, third paragraph); however, this does not support the phrase "are not sites having structural/functional importance to proper functioning of the polymerizing agent" which differs in scope. Written basis is provided for "cysteine residue includes a tag covalently bonded thereto through the SH group"; however this does not provide adequate written support for "is covalently bonded to the cysteine through its SH group" which is broader in scope as it does not state what is covalently bonded to the cysteine or whose "SH group".

Because the introduction of "and where the polymerizing agent lacks 3' to 5' exonuclease activity" (claim 10), "lacking 3' to 5' exonuclease activity" (claims 13-14), "the polymerizing agent lacking 3' to 5' exonuclease activity" (claim 79), "where the site comprises a naturally occurring cysteine site or a cysteine replacement site in the polymerizing agent selected so that the site is less than or equal to about 25Å from a tag on each incorporating monomer regions and are not sites having structural/functional importance to proper functioning of the polymerizing agent and is covalently bonded to the cysteine through its SH group" (claim 79), "the site comprises a naturally occurring cysteine site or a cysteine replacement site in the

> polymerizing agent selected so that the site is less than or equal to about 25Å from a tag on each incorporating monomer regions and is covalently bonded to the cysteine through its SH group" (claim 89), "the site is less than or equal to about 15Å from a tag on each incorporating monomer" (claims 80 and 90), and "the site is less than or equal to about 10Å from a tag on each incorporating monomer" (claims 81 and 91) lack written basis, filed on 8/22/06, these phrases are considered to be NEW MATTER. Claims 15-19, 82-88 and 92-99 are also rejected due to their direct or indirect dependency from claims 10, 13, 14, 79, and 89. This rejection is necessitated by amendment.

Although Applicants have canceled several claims such as claims 14 and 15 for a more expeditious examination of this application, Applicants reserve the right and have filed a number of divisional/continuation applications to reassert this subject matter.

Lacking 3' to 5' Exonuclease Activity

Applicants respectfully disagree with the Examiner. The present invention is clear; the polymerizing agents lack 3' to 5' exonuclease activity to avoid problematic errors during nucleic acid sequencing. The reason *Taq* was chosen as a model DNA polymerase is because it inherently lacks 3' to 5' exonuclease activity so that aspects of the invention could be verified experimentally. Further, the section pertaining to Enzyme Choice, where selecting a polymerizing agent such as a polymerase for single molecule sequencing is discussed, states as its heading that the polymerizing agent is free from 3' to 5' exonuclease activity, and the first paragraph in this part of the disclosure ends by stating that, "*Taq* DNA polymerase, sometimes simply referred to herein as *Taq*, has many attributes that the inventors can utilize in constructing tagged polymerases for use in the inventions disclosed in the application. Of course, **ordinary artisans will recognize that other polymerases can be adapted for use in the single-molecule sequencing system of this invention.**" Specification at page 58 second paragraph (emphasis added). This heading makes it very clear that this teaching was directed to all polymerizing agents used in the practice of invention. The following excerpts from the specification support and make it clear that DNA polymerases **lacking** 3' to 5' exonuclease active are required and DNA polymerase **having** 3' to 5' exonuclease activity make sequencing problematic:

> **The Polymerase Is Free from 3' to 5' Exonuclease Activity**
>
> The *Taq* DNA polymerase is does not contain 3' to 5' exonuclease activity, which means that the polymerase cannot replace a base, for which fluorescent signal was detected, with another base which would produce another signature fluorescent signal.
>
> All polymerases make replication errors. The 3' to 5' exonuclease activity is used to proofread the newly replicated DNA strand. Since *Taq* DNA polymerase lacks this proofreading function, an error in base incorporation becomes an error in

DNA replication. Error rates for *Taq* DNA polymerase are 1 error per ~100,000 bases synthesized, which is sufficiently low to assure a relatively high fidelity. See, *e.g.*, Eckert and Kunkel, 1990; Cline *et al.*, 1996. It has been suggested and verified for a polymerase that the elimination of this exonuclease activity uncovers a decreased fidelity during incorporation. Thus, *Taq* polymerase must - by necessity - be more accurate during initial nucleotide selection and/or incorporation, and is therefore an excellent choice of use in the present inventions.

The error rate of engineered polymerases of this invention are assayed by determining their error rates in synthesizing known sequences. The error rate determines the optimal number of reactions to be run in parallel so that sequencing information can be assigned with confidence. The optimal number can be 1 or 10 or more. For example, the inventors have discovered that base context influences polymerase accuracy and reaction kinetics, and this information is used to assign confidence values to individual base calls. However, depending on the goal of a particular sequencing project, it may be more important to generate a genome sequence as rapidly as possible. For example, it may be preferable to generate, or draft, the genome sequence of a pathogen at reduced accuracy for initial identification purposes or for fast screening of potential pathogens.

Assay Fidelity of γ-phosphate Tagged Nucleotide Incorporation

The *Taq* DNA polymerase lacks 3' to 5' exonuclease activity (proofreading activity). If **the polymerase** used in single-molecule DNA sequencing possessed a 3' to 5' exonuclease activity, the polymerase would be capable of adding another base to replace one that would be removed by the proofreading activity. **This newly added base would produce a signature fluorescent signal evidencing the incorporation of an additional base in the template, resulting in a misidentified DNA sequence, a situation that would render the single-molecule sequencing systems of this invention problematic.**

Specification at page 58 second paragraph (emphasis added, note that the bold "**the polymerase**" in the second sentence clearly generalizes this criterion).

These quotes make it abundantly clear that the presence of 3' to 5' exonuclease activity would make single molecule sequencing "problematic" based on detecting changes in a detectable property during each of a sequence of dNTP incorporations. Using polymerases that lack 3' to 5' exonuclease activity remove this problematic outcome. One of ordinary skill in the art would clearly recognize this teaching as sufficient to enable the use in single molecule DNA sequencing of polymerases that lack 3' to 5' exonuclease activity; otherwise the sequencing results would be problematic and incapable of ready sequence identification.

The application in the entire section dealing with polymerase selection clearly teaches that *Taq* is an illustrative example of the type of polymerases – ones that lack 3' to 5' exonuclease activity.

Moreover, *Taq* was also used to illustrate amino acid residue selection for cysteine mutagenesis because *Taq* also lacks any cysteine residues.

To an ordinary artisan, the section makes it abundantly clear that the specification fully enables the limitation that polymerizing agents lack 3' to 5' exonuclease activity. Using such polymerizing agents allows sequencing to be simplified in that multiple signals per nucleotide site are not observed and that naturally occurring cysteine residues or cysteine residues added by site specific mutagenesis can be used to attach tags to selected polymerase sites.

Cysteine Site or a Cysteine Replacement Site and Distance

The following quotes make it clear the *Taq* was an illustrative example for using cysteine mutagenesis to construct polymerases capable of accepting a tag at a site on the polymerase selected from sites that undergo considerable motion as the polymerase transition from its open form to its closed form. It is also clear from the following specification excerpts that the distances set forth in the specification relate to the distance from the tag on the polymerase to the tag on a tagged dNTP when the tagged dNTP is undergoing incorporation.

> The resulting mutant *Taq* polymerases are then reacted with a desired atomic or molecular tag **to tag the cysteine in the mutant structure through the SH group of the cysteine residue** and screened for native and/or tagged dNTP incorporation and incorporation efficiency. The mutant polymerases are also screened for fluorescent activity during base incorporation. Thus, the present invention also relates to mutant Taq polymerase having a cysteine residue added one or more of the sites selected from the group consisting of 513-518, 643, 647, 649 and 653-661. **After cysteine replacement and verification of polymerase activity using the modified dNTPs, the mutant *Taq* polymerases are reacted with a tag through the SH group of the inserted cysteine residue.**

Specification at page 67 (emphasis added).

> The present invention provides cooperatively tagged polymerizing agents and tagged monomers, where a detectable property of at least one of the tags changes **when the tags are within a distance sufficient to cause a measurable change in the detectable property. If the detectable property is fluorescence induced in one tag by energy transfer to the other tag or due to one tag quenching the fluorescence of the other tag or causing a measurable change in the fluorescence intensity and/or frequency, the measurable change is caused by bringing the tags into close proximity to each other, *i.e.*, decrease the distance separating the tags.** Generally, the **distance** needed to cause a measurable change in the detectable property is **within** (less than or equal to) **about 100Å**, preferably **within about 50Å**, particularly **within about 25Å**, especially **within about 15Å** and most preferably **within about 10Å**. Of course, one skilled in the art will recognize that a distance sufficient to cause a measurable change in a detectable property of a tag will depend on many parameters including the location of the tag, the nature of the tag, the

solvent system, external fields, excitation source intensity and frequency band width, temperature, pressure, *etc.*

Specification at page 18-19 (emphasis added).

The present invention provides cooperatively tagged depolymerizing agents and tagged polymers, where a detectable property of at least one of the tags changes when the tags are within a distance sufficient to cause a change in measurable change in the detectable property. If the detectable property is fluorescence induced in one tag by energy transfer to the other tag or due to one tag quenching the fluorescence of the other tag or causing a measurable change in the fluorescence intensity and/or frequency, the measurable change is caused by bringing to [two] tags into close proximity to each other, *i.e.*, **decrease the distance separating the tags**. Generally, the distance needed to cause a measurable change in the detectable property is **within** (less than or equal to) **about 100Å**, preferably **within about 50Å**, particularly **within about 25Å**, especially **within about 15Å** and most preferably **within about 10Å**. Of course, one skilled in the art will recognize that a distance sufficient to cause a measurable change in a detectable property of a tag will depend on many parameters including the location of the tag, the nature of the tag, the solvent system, external fields, excitation source intensity and frequency band width, temperature, pressure, *etc.*

Specification at page 19 (emphasis added).

The present invention provides cooperatively tagged polymerase and tagged monomers, where a detectable property of at least one of the tags changes when the tags are within a distance sufficient or in close proximity to cause a measurable change in the detectable property. If the detectable property is fluorescence induced in one tag by energy transfer to the other tag or due to one tag quenching the fluorescence of the other tag or causing a measurable change in the fluorescence intensity and/or frequency, **the measurable change is caused by bringing to [two] tags into close proximity to each other, *i.e.*, decrease the distance separating the tags. Generally, the distance or close proximity is a distance between about 100Å and about 10Å. Alternatively, the distance is less than or equal to about 100Å, preferably less than or equal to about 50Å, particularly less than or equal to about 25Å, especially less than or equal to about 15Å and most preferably less than or equal to about 10Å.** Of course, one skilled in the art will recognize that a distance sufficient to cause a measurable change in a detectable property of a tag will depend on many parameters including the location of the tags, the nature of the tags, the solvent system (polymerizing medium), external fields, excitation source intensity and frequency band width, temperature, pressure, *etc.*

Specification at page 20 (emphasis added).

<u>No Cysteines Are Present in the Protein Sequence</u>

The *Taq* DNA polymerase contains no cysteines, which allows the easy generation of cysteine-containing mutants in which a single cysteine is placed or substituted for an existing amino acid at strategic sites, **where the inserted cysteine serves as a tag attachment site.**

Specification at page 39 (emphasis added).

> **In one preferred embodiment, a fluorescence donor is attached to a site on the polymerase comprising a replaced amino acid more amenable to donor attachment such as cysteine and four unique fluorescence acceptors are attached to each dNTP.** For example, fluorescein is attached to a site on the polymerase and rhodamine, rhodamine derivatives and/or fluorescein derivatives are attached to each dNTP. **Each donor-acceptor fluorophore pair is designed to have an absorption spectra sufficiently distinct from the other pairs to allow separate identification after excitation.** Preferably, the donor is selected such that the excitation light activates the donor, which then efficiency transfers the excitation energy to one of the acceptors. After energy transfer, the acceptor emits it unique fluorescence signature. The emission of the fluorescence donor must significant overlap with the absorption spectra of the fluorescence acceptors for efficient energy transfer. However, the methods of this invention can also be performed using two, three or four unique fluorescence donor-acceptor pairs, by running parallel reactions.

Specification at page 43 (emphasis added).

> **In general, the polymerase is tagged by replacing a selected amino acid codon in the DNA sequence encoding the polymerase with a codon for an amino acid that more easily reacts with a molecular tag such as cysteine via mutagenesis.** Once a mutated DNA sequence is prepared, the mutant is inserted into *E. coli* for expression. After expression, the mutant polymerase is isolated and purified. The purified mutant polymerase is then tested for polymerase activity. After activity verification, the mutant polymerase is reacted with a slight molar excess of a desired tag to achieve near stoichiometric labeling. Alternatively, the polymerase can be treated with an excess amount of the tag and labeling followed as a function of time. The tagging reaction is than stopped when near stoichiometric labeling is obtained.

Specification at page 45 (emphasis added).

Applicants in the text of the specification from page 45 to page 53 outlined the procedure used to mutate sequences for encoding polymerases, using *Taq* as a model, to add cysteine codon for tag attachment. The text clearly sets forth the criteria for effective tag placement on a polymerase or any other polymerizing agent. Again, it is clear from the manner in which the specification was constructed and on its basic teaching that the inventors used *Taq* as a model for constructing appropriately tagged polymerizing agents and that such teaching is fully enabling.

All broad statements throughout the specification are directed to polymerizing agents for single molecule sequencing, in general, and only to *Taq* as a specific example. All broad statements throughout the specification then list polymerizing agents that will work in the invention, such as many well-known polymerases. One of ordinary skill in the art would understand that this site selection teaching is true for any polymerase or other polymerizing agent to be used in single molecule sequencing of this invention. There is no teaching that the criteria are exclusive for *Taq*. In fact, the exact opposite is true – the inventors laid out a very specific process for selecting sites

and for mutating those sites to a cysteine and for tagging the cysteine sites through the cysteine's SH group, regardless of the polymerizing agent.

Additionally, Applicants taught how one, using competitive chemistry, could tag one cysteine site relative to another if more than one cysteine site was present in the polymerase. **This teaching clearly could not have been directed to *Taq* as *Taq* does not have any naturally occurring cysteines:**

> In general, the polymerase is tagged by replacing a selected amino acid codon in the DNA sequence encoding the polymerase with a codon for an amino acid that more easily reacts with a molecular tag such as cysteine via mutagenesis. Once a mutated DNA sequence is prepared, the mutant is inserted into *E. coli* for expression. After expression, the mutant polymerase is isolated and purified. The purified mutant polymerase is then tested for polymerase activity. After activity verification, the mutant polymerase is reacted with a slight molar excess of a desired tag to achieve near stoichiometric labeling. Alternatively, the polymerase can be treated with an excess amount of the tag and labeling followed as a function of time. The tagging reaction is than stopped when near stoichiometric labeling is obtained.
>
> If the mutant polymerase includes several sites including the target residue that can undergo tagging with the desired molecular tag, then the tagging reaction can also be carried out under special reaction conditions such as using a protecting group or competitive inhibitor and a reversible blocking group, which are later removed. If the target amino acid residue in the mutant polymerase is close to the active dNTP binding site, a saturating level of a protecting group or a competitive inhibitor is first added to protect the target residue and a reversible blocking group is subsequently added to inactivate non-target residues. The protecting group or competitive inhibitor is then removed from the target residue, and the mutant polymerase is treated with the desired tag to label the target residue. Finally, the blocking groups are chemically removed from non-target residues in the mutant polymerase and removed to obtain a tagged mutant polymerase with the tag substantially to completely isolated on the target residue.
>
> Alternatively, if the target residue is not near the active site, the polymerase can be treated with a blocking group to inactivate non-target residues. After removal of unreacted blocking group, the mutant polymerase is treated with the desired tag for labeling the target residue. Finally, the blocking groups are chemically removed from the non-target residues in the mutant polymerase and removed to obtain the tagged mutant polymerase.

Specification at page 46.

Applicants also clearly set forth the distance criteria, both needed and preferred, for the distance between the tag on the polymerase and the tag on an incorporating dNTP, during incorporation. The tag distances for tagged polymerases and incorporating tagged dNTPs are set forth repetitively throughout the specification. Sometimes these distances are expressed in Å and sometimes as a ratio of FRET variables. Regardless of the exact context, the specification is clear;

the tag interaction is to occur at a close proximity, which is a distance between about 100Å and about 10Å, and preferably within about 50Å, particularly within about 25Å, especially within about 15Å and most preferably within about 10Å.

The inclusion of any one of these distance limitations clearly is not new matter as the application is replete with recitations of these limitations. These distance limitations are specifically set forth to maximize tag-tag interaction during nucleotide incorporation by insuring the inter-tag distance is either between about 100Å and about 10Å, or preferably within about 50Å, particularly within about 25Å, especially within about 15Å and most preferably within about 10Å.

Because Applicants believe that the specification: (1) clearly enables and supports polymerizing agents and particularly polymerases that lack 3' to 5' exonuclease activity, where these latter polymerases avoid problematic results, (2) clearly enables and supports cysteine residues as the sites for tag attachment, regardless of whether the cysteine is naturally occurring or mutated or engineered into the polymerase, and (3) clearly enables and supports the distance limitations between a tag on the polymerase and a tag on an incorporating dNTP, Applicants respectfully request withdrawal of these section 112, first paragraph rejections.

Claim Rejections - 35 USC § 112, Second Paragraph

Claims 79 and 89 (last line of each) recite the limitation "the cysteine".

The Examiner contends as follows:

> There is insufficient antecedent basis for this limitation in these claims. While there is prior mention of a cysteine site or cysteine replacement site, there is not previous mention of an actual cysteine. Clarification of this issue via clearer claim wording is requested. Claims 80-88 and 90-99 are also rejected due to their dependency from claims 79 and 89.

Applicants have amended claims 79 and 89 to alter the cysteine reference to the site reference introduced earlier in the claim. Applicants, therefore, respectively request withdrawal of this section 112, second paragraph rejection.

Claims 79 and 89 recite the phrase "is covalently bonded to the cysteine through its SH group" (last line) which is vague and indefinite.

The Examiner contends as follows:

> It is unclear what is covalently bonded to the cysteine which may be the polymerizing agent, the site, or the tag. It is unclear if the "its" is referring to the cysteine, polymerizing agent, or the site. Clarification of this issue via clearer claim

wording is requested. Claims 80-88 and 90-99 are also rejected due to their dependency from claims 79 and 89.

Applicants have added the missing language to clarify that the polymerizing agent tag "is covalently bonded to the cysteine through its SH group." Applicants, therefore, respectively request withdrawal of this section 112, second paragraph rejection.

Claim Rejections - 35 USC § 102

Claims 10, 13-18, 50-55, 57-62, 64-69, and 71-77 stand rejected under 35 U.S.C. 102(e) as being anticipated by Korlach et al. (US 2006/0078937 Al) in light of Wisniewski et al. (Journal of Biological Chemistry, 1999, Volume 274, Number 40, pages 28175-28184) and Gardner et al. (Nucleic Acids Research, 2002, Volume 30, Number 2, pages 605-613). This rejection is necessitated by amendment.

The Examiner contends as follows:

> Korlach et al. disclose a composition comprising a polymerizing agent including a molecular tag covalently bonded to a site on the polymerizing agent and a monomer including a molecular tag, where at least one of the tags has a fluorescence property that undergoes a change before, during and/or after each of a sequence of monomer incorporations due to an interaction between the polymerizing agent tag and the monomer tag (claim 62), as stated in instant claim 10. Korlach et al. disclose a composition wherein the polymerizing agent is a polymerase or reverse transcriptase (claim 63), as stated in instant claims 13 and 72. Korlach et al. disclose a composition wherein the polymerase is selected from the group of Taq DNA polymerase, T7 DNA polymerase, Sequenase, and the Klenow fragment from E. coli DNA polymerase (claim 64), as stated in instant claims 14 and 73. Korlach et al. disclose a composition wherein the reverse transcriptase comprises HIV reverse transcriptase (claim 65), as stated in instant claims 15 and 74. Korlach et al. disclose a composition wherein each of the monomers comprises a deoxynucleotide triphosphate (dNTP) and the monomer tag is covalently bonded to the α, β, or γ-phosphate group of each dNTP (claim 66 and paragraphs 0064-0067), as stated in instant claim 16 and the terminal phosphate of the monomer, as stated in instant claims 71 and 75. Korlach et al. disclose a composition wherein the tags comprise fluorescent tags, and the fluorescence property comprises an intensity, wavelength, and/or frequency of emitted fluorescent light (claim 67), as stated in instant claims 17 and 76. Korlach et al. disclose a composition wherein the fluorescence property is fluorescence resonance energy transfer (FRET) where either the monomer tag of the polymerase tag comprises a donor and the other tag comprises an acceptor and where FRET occurs when the two tags are in close proximity (claim 68), as stated in instant claims 18 and 77. Korlach et al. disclose a composition wherein the polymerase comprises *Taq* DNA Polymerase having a tag attached to an amino acid position at a specific amino acid of the *Taq* DNA polymerase that is less than 60Å from an incorporating nucleotide (claim 69). Korlach et al. disclose a composition

comprising a polymerizing agent including a molecular tag covalently bonded to a site on the polymerizing agent and a deoxynucleotide triphosphate (dNTP) including a molecular tag covalently bonded to the (β, or γ-phosphate group of the dNTP, where at least one of the tags has a fluorescence property that undergoes a change before, during and/or after each of a sequence of monomer incorporations due to an interaction between the polymerizing agent tag and the monomer (claim 70), as stated in instant claims 50, 57, and 64. Korlach et al. disclose a composition wherein the polymerizing agent is a polymerase or reverse transcriptase (claim 71), as stated in instant claims 51, 58, and 65. Korlach et al. disclose a composition wherein the polymerase is selected from the group consisting of Taq DNA polymerase, T7 DNA polymerase, Sequenase, and the Klenow fragment from E. coli DNA polymerase (claim 72), as stated in instant claims 52, 59, and 66. Korlach et al. disclose a composition wherein the reverse transcriptase comprises HIV reverse transcriptase (claim 73), as stated in instant claims 53, 60, and 67. Korlach et al. disclose a composition wherein the tags comprise fluorescent tags, and the fluorescence property comprises an intensity, wavelength, and/or frequency of emitted fluorescent light (claim 74), as stated in instant claims 54, 61, and 68. Korlach et al. disclose a composition wherein the fluorescence property is fluorescence resonance energy transfer (FRET) where either the monomer tag of the polymerase tag comprises a donor and the other tag comprises an acceptor and where FRET occurs when the two tags are in close proximity (claim 75), as stated in instant claims 55, 62, and 69. Korlach et al. disclose a composition wherein the polymerase comprises *Taq* DNA Polymerase having a tag attached to an amino acid position at a specific amino acid of the *Taq* DNA Polymerase, that is less than 60 A from an incorporating nucleotide (claim 76). Korlach et al. disclose the sequencing method of the present invention can be carried out using polymerase and no exonuclease (paragraph 0019). It is noted that Korlach et al. do not specifically state the inherent characteristics of HIV-1 reverse transcriptase, Sequenase, and Taq DNA polymerase I in that they lack 3' to 5' exonuclease activities. Wisniewski et al. (page 28175, col. 2, first paragraph) and Gardner et al. (page 606, col. 1, last paragraph) recite these inherent chararacteristics. Wisniewski et al. and Gardner et al. are not being used as prior art, but rather to document these inherent characteristics of HIV-1 reverse transcriptase, Sequenase, and Taq DNA polymerase I.

Korlach et al. in light of Wisniewski et al. and Gardner et al. anticipate the limitations in claims 10, 13-18, 50-55, 57-62, 64-69, and 71-77.

General Comments

Applicants respectfully disagree with the Examiner's statements that Korlach et al. disclosed polymerizing agents. While it is true the Korlach et al. did disclose polymerases and reverse transcriptases, Korlach et al. never used the term "polymerizing agent" in their original application. This term, as used in the present invention, refers to any agent that can step-wise add monomers to form a sequence specific oligomer or polymer. In fact, Applicants defined the term polymerizing agents as follows:

> Suitable polymerizing agents for use in this invention include, without limitation, any polymerizing agent that polymerizes monomers relative to a specific template such as a DNA or RNA polymerase, reverse transcriptase, or the like or that polymerizes monomers in a step-wise fashion.

Moreover, the Korlach et al. specification also never uses the term monomer. While nucleotides are monomers, the definition of monomer in the Applicants invention is much broader than a nucleotide and encompasses any molecular that can be polymerized by a polymerizing agent.

> The term monomer as used herein means any compound that can be incorporated into a growing molecular chain by a given polymerase. Such monomers include, without limitations, naturally occurring nucleotides (*e.g.*, ATP, GTP, TTP, UTP, CTP, dATP, dGTP, dTTP, dUTP, dCTP, synthetic analogs), precursors for each nucleotide, non-naturally occurring nucleotides and their precursors or any other molecule that can be incorporated into a growing polymer chain by a given polymerase. Additionally, amino acids (natural or synthetic) for protein or protein analog synthesis, mono sacchairides for carbohydrate synthesis or other monomeric syntheses.

Thus, Applicants believe that the Examiner is misconstruing the teaching of Korlach et al., and that the claims of the cited Korlach et al. are not supported by the Korlach et al. specification.

Applicants respectfully remind the Examiner, that this rejection must be confined to the teaching in the original Korlach et al. application and not to the claims of the cited Korlach et al. application. The claims in the cited Korlach et al. application were not part of the claim set of the original Korlach et al. application as filed on 17 May 2000, and they cannot be relied on by the Examiner to support a rejection of the claims of Applicants' application, **because they are not supported in the original Korlach et al. application.**

Further, in contrast to the Remarks/Arguments submitted with Korlach et al. 11/21/2005 Continuation filing, these claims also find absolutely no support in the Korlach et al. 1999 provisional document from which the Korlach et al. application claims priority. In particular, the 1999 provisional includes absolutely no reference to beta or gamma phosphate labeled nucleotides. The beta or gamma phosphate labeled nucleotide disclosure is only present in the 2000 filing. Dr. Hardin's Rule 131 declaration and attached documents clearly support the fact that Applicants can prove an earlier invention date than the 17 May 2000 Korlach et al. filing, as it relates to the use of non-persistently labeled nucleotides (*e.g.*, phosphates and specifically gamma phosphate labeled nucleotides) in nucleic acid sequencing.

Regardless of Applicants ability to antedate the cited Korlach et al. reference for the use of non-persistently labeled nucleotides in single molecule sequencing, Applicants will show that the

cited Korlach et al. reference is not entitled to any of the claims in the cited Korlach et al. continuation application specifically relied on by the Examiner, as they find no support in the original Korlach et al. specification and were not part of the original claim set as filed. Applicants will also show that the teaching of Korlach et al. does not anticipate or render obvious any of the claims of the present application.

Claims 10 and 13-18

Applicants have canceled claims 14 and 15, without prejudice. Applicants set forth above a clear statement of support for the limitation that the polymerizing agent lack 3' to 5' exonuclease activity, as the presence of this activity would render base identification – sequencing – problematic. Korlach et al. does not disclose a limitation for polymerizing agent that lack 3' to 5' exonuclease activity regardless of the Examiner's attempts to imply this limitation due to the fact that certain polymerizing agents normally lack 3' to 5' exonuclease activity. The Examiner relies on inherency to render this limitation anticipated, but does not speak to the fact that Korlach et al. teaches many other polymerases that include 3' to 5' exonuclease activity and there is no teaching that such polymerases would lead to problematic sequencing information. This activity would allow a nucleotide once incorporated to be removed and replaced. Thus, the sequence read out would be incorrect whenever a nucleotide was removed and replaced (an apparent occurrence of two nucleotides, whereas only one exists in the template sequence). Applicant's present claim set includes this limitation, a limitation supported by the Applicants' specification and not supported in the cited Korlach et al. reference. In fact, Schneider et al. disclose using "the *E. coli* DNA polymerase I, specifically the Klenow fragment which has 3' to 5' exonuclease activity."

While Applicants note the following statement by Korlach et al.:

> In addition, the sequencing method of the present invention can be carried out using polymerase and no exonuclease. This results in greater simplicity, easier miniaturizability, and compatibility to parallel processing of a single-step technique.

This statement did not relate to the nature of the polymerase concerning inherent exonuclease activity, 3' to 5' or 5' to 3'; the statement related to the benefits of a sequencing strategy based on a step-by-step addition of nucleotides as opposed to a step-by-step removal of nucleotides. This interpretation is further supported by the following paragraph in their specification discussing processivity of polymerases versus exonucleases.

Irrespective of the inherent properties of several of the polymerases the Examiner selectively extracted from Korlach et al., Korlach et al. disclosed many polymerases that include 3' to 5'

exonuclease activity and used polymerases that have this activity in their examples. Thus, because Korlach et al.do not disclose the limitation that the polymerases used in single molecule sequencing lack 3' to 5' exonuclease activity, Korlach et al. cannot anticipate the present claims including this limitation. Applicants, therefore, respectfully request withdrawal of this rejection.

Claims 50-55, Claims 57-62, Claims 64-69 and Claims 71-77

First, Applicants want to make it very clear that Korlach et al. do not disclose a composition, a method or an apparatus, where sequence information is determined from an interaction between a labeled polymerase and a beta and/or gamma labeled nucleotide. Second, the Examiner cannot rely on claims 62-77 of this Korlach et al. continuation application (11/285422) because these added claims were not part of the original 17 May 2000 application (specification and original claims) claiming priority to the 1999 provisional and these added claims find no support in the origin 17 May 2000 Korlach et al. specification. Therefore, the Examiner can only rely on the original 17 May 2000 Korlach et al. specification and original claims to mount 102 or 103 challenges against the present application, because only subject matter of the original 17 May 2000 Korlach et al. filing may be used to support claim rejections. **Therefore, Applicants will direct all arguments to the Korlach et al. specification and not to the added claims relied on by the Examiner, because they do not make up part of the original specification or original claims and find no support in the specification. Further, all Korlach et al. applications that claim an invention that detects sequencing data due to an interaction between a labeled polymerase and a beta or gamma labeled nucleotide must be considered CIP and not permitted reliance on the 1999 or 2000 filing dates.**

As stated, the 1999 Korlach et al. filing is silent with respect to beta or gamma labeled nucleotides. The **only** beta or gamma labeled dNTP disclosure in the 17 May 2000 Korlach et al specification (outside of the original claim set, which does disclose beta or gamma labeled dNTPs, but the claims are not linked to an interactive sequencing strategy) reads as follows:

> [0067] In addition to fluorescent labels that remain in the nucleic acid during synthesis, nucleotides that are labelled fluorescently or otherwise and carry the label attached to either the beta or gamma phosphate of the nucleotide can also be used in the sequencing procedure of the present invention. Analogous compounds have previously been synthesized in the form of NTP analogs and have been shown to be excellent substrates for a variety of enzymes, including RNA polymerases. See Yarbrough et al., "Synthesis and Properties of Fluorescent Nucleotide Substrates for DNA-dependent RNA Polymerase," Journal of Biological Chemistry 254:12069-12073 (1979), and Chatterji et al., "Fluorescence Spectroscopy Analysis of Active and Regulatory Sites of RNA Polymerase," Methods in Enzymology 274: 456-479

(1996), which are hereby incorporated by reference. During the synthesis of DNA, the bond cleavage in the nucleotide occurs between the alpha and the beta phosphate, causing the beta and gamma phosphates to be released from the active site after polymerization, and the formed pyrophosphate subsequently diffuses or is convected away from the nucleic acid. In accordance with the present invention, it is possible to distinguish the event of binding of a nucleotide and its incorporation into nucleic acid from events just involving the binding (and subsequent rejection) of a mismatched nucleotide, because the rate constants of these two events are drastically different. The rate-limiting step in the successive elementary steps of DNA polymerization is a conformational change of the polymerase that can only occur after the enzyme has established that the correct (matched) nucleotide is bound to the active site. Therefore, an event of a mismatched binding of a nucleotide analog will be much shorter in time than the event of incorporation of the correct base. See Patel et al., "Pre-Steady-State Kinetic Analysis of Processive DNA Replication Including Complete Characterization of an Exonuclease-Deficient Mutant," Biochemistry 30: 511-525 (1991) and Wong et al., "An Induced-Fit Kinetic Mechanism for DNA Replication Fidelity: Direct Measurement by Single-Turnover Kinetics," Biochemistry 30: 511-525 (1991), which are hereby incorporated by reference. As a result, the fluorescence of the label that is attached to the beta or gamma phosphate of the nucleotide analog remains proximate to the polymerase for a longer time in case the nucleotide analog is polymerized, and can be distinguished in accordance to the scheme described above for FIG. 3. After incorporation, the label will diffuse away with the cleaved pyrophosphate. This procedure is shown in FIG. 4. FIG. 4A shows the structure of 1-aminonaphthalene-5-sulfonate (AmNS)-dUTP, a representative example of a nucleotide analog carrying a fluorescent label attached to the gamma phosphate, with the cleavage position indicated by the dashed line. FIG. 4B-D show the successive steps of incorporation and release of the pyrophosphate-fluorophore complex, in analogy to FIG. 2. The time trace of fluorescence for this scheme will be the same as shown in FIG. 3. Thus, this is an alternative scheme to the one outlined above in which the fluorophore is first incorporated into the nucleic acid and the signal is subsequently eliminated by photobleaching or photochemical cleavage after identification of the label.

[0068] The identification of the particular fluorescently labelled nucleotide analog that is incorporated against the background of unincorporated nucleotides diffusing or flowing proximally to the nucleic acid polymerizing enzyme can be further enhanced by employing the observation that for certain fluorescently labelled dNTPs (e.g., coumarin-5-dGTP, or AmNS-UTP), the presence of the base in the form of a covalent linkage significantly reduces (i.e. quenches) the fluorescence of the label. See Dhar et al., "Synthesis and Characterization of Stacked and Quenched Uridine Nucleotide Fluorophores," Journal of Biological Chemistry 274: 14568-14572 (1999), and Draganescu et al., "Fhit-Nucleotide Specificity Probed with Novel Fluorescent and Fluorogenic Substrates," Journal of Biological Chemistry 275: 4555-4560 (2000), which are hereby incorporated by reference. The interaction between the fluorophore and the base quenches the fluorescence, so that the molecule is not very fluorescent in solution by itself. However, when such a fluorescent nucleotide is incorporated into the nucleic acid, the fluorophore gets disconnected from the nucleotide and the fluorescence is no longer quenched. For the case of a linkage to

> the beta or gamma phosphate of the nucleotide, this occurs naturally through the enzymatic activity of the polymerase, in the case of fluorophores linked to the base, this would have to be accomplished by photochemical cleavage. The signal of fluorescence from the cleaved fluorophore is much brighter and can be detected over the possible background of the plurality of quenched molecules in the vicinity of the polymerase/nucleic acid complex.

Korlach et al 2006/0078937 – this specification is identical to the 17 May 2000 Korlach et al. filing.

The beta or gamma labeled disclosure did not disclose the use of beta or gamma labeled dNTPs in a sequencing strategy involving an interaction between a labeled dNTP and a labeled polymerase. Nowhere in the Korlach et al. specification is there any disclosure or teaching consistent with sequencing based on interactions between a labeled polymerizing agent and a beta or gamma labeled nucleotide. In fact, the only mention of a strategy involving interactions between a labeled polymerizing agent and labeled nucleotides reads as follows:

> [0104] Detection of fluorescence resonance energy transfer (FRET) from a donor fluorophore (e.g., a donor attached to the polymerase) to adjacent nucleotide analog acceptors that are incorporated into the growing nucleic acid strand suggests a further elegant possibility of lowering background from incorporated nucleotides. FRET only reaches very short distances including about 20 nucleotides and decays at the reciprocal sixth power of distance. The excited donor molecule transfers its energy only to nearby acceptor fluorophores, which emit the spectrally resolved acceptor fluorescence of each labelled nucleotide as it is added. Already incorporated nucleotides farther away from the donor would not contribute to the fluorescent signal since distance and orientation constraints of energy transfer reduce the effective range of observation to less than 60Å, thereby effectively eliminating background fluorescence from unincorporated nucleotides. **Without photobleaching, the method requires high sensitivity since repeat nucleotides leave the range of FRET at the same rate that new nucleotides are added, possibly creating sequence recognition ambiguity. Photobleaching or photochemical cleavage, or their combination as discussed above could resolve the problem.** Photobleaching of the donor molecules using FRET can be avoided if it is the template nucleic acid that is attached and the donor bearing nucleic acid polymerizing enzyme is periodically replaced.

Korlach et al 2006/0078937 (emphasis added) – this specification is identical to the 17 May 2000 Korlach et al. filing.

Several points are worth noting. Again, nowhere does Korlach et al. disclose the use of beta or gamma labeled dNTPs in an interactive strategy such as a FRET strategy. Clearly, Korlach et al. disclosed that in a FRET strategy the labels would have to be photobleached or photocleaved after incorporation, or they labels now on the resulting DNA strand would contribute to the fluorescent

spectra until the nucleotide bearing the label moved outside of the 60Å effective FRET distance by subsequence nucleotide incorporations.

Applicants respectfully disagree with the Examiner's reading of Korlach et al. as it pertains to the 60Å teaching. Korlach et al. **did not disclose the distance between the label on the polymerase and the label on the nucleotide except in the context of the FRET distance needed to no longer see a label on a previously incorporated nucleotide. Korlach et al. is non-enabling for any other reading of this teaching.**

Additional problems exist even for their base labeled dNTP-polymerase strategy eluded to in the above quoted paragraph [0104]. The problems with this teaching are that: 1) it does not disclose anything about the tag location on the polymerase; 2) it does not disclose anything specific about the distance between the tag on the polymerase and the tag on the incorporating nucleotide; and 3) it does not disclose anything about the requirement that the polymerase tag location not interfere with nucleotide incorporation, protein function and/or protein folding. An ordinary artisan may be able to infer that the polymerase label is positioned such as to not interfere with base incorporation, but Korlach et al. give absolutely no guidance for how one would select a site on the polymerase for attaching a label or how one would go about performing the attachment. Additionally, tagging of a polymerase is not a straight forward affair, without guidance an ordinary artisan is faced with a daunting task.

Clearly, Korlach et al. did not disclose the use of beta or gamma labeled dNTPs in an interactive strategy such as a FRET strategy utilizing a labeled polymerase. If they had, they would have stated that using beta or gamma labeled dNTPs would obviate the necessity for photobleaching or photochemical cleavage of the labels on the incorporated dNTPs in this context. Korlach et al. include no such teaching. As the use of beta or gamma labeled dNTPs would greatly simplify the problem associated with removing labels and/or waiting for labels to move out of the FRET observation distance, the lack of any disclosure of this fact is clear evidence that the Korlach et al. did not and does not disclose, teach or even suggest such a strategy. Further, Korlach et al. stated:

> **Without photobleaching, the method requires high sensitivity since repeat nucleotides leave the range of FRET at the same rate that new nucleotides are added, possibly creating sequence recognition ambiguity. Photobleaching or photochemical cleavage, or their combination as discussed above could resolve the problem.**

This statement clearly shows that Korlach et al. only disclosed and taught persistently labeled dNTP in their FRET sequencing strategy, *i.e.*, dNTPs labeled on the base, sugar or backbone (alpha) phosphate.

Moreover, **Schneider et al. more clearly disclosed the use of labeled polymerases and labeled nucleotides in a FRET sequencing strategy, *but made absolutely no mention of beta or gamma labeled dNTPs whatsoever*.** Rather their invention focused solely on nucleotides labeled on the base, sugar or backbone phosphate, *i.e.*, persistently labeled nucleotides.

Although Korlach et al. disclosed beta or gamma labeled dNTPs, while Schneider et al. did not; neither Korlach et al. nor Schneider et al., two groups of experts in this area, disclosed a label interactive strategy involving detecting interactions between beta or gamma labeled dNTPs and a labeled polymerase, regardless of whether the interaction is FRET or direct detection of the fluorescent properties of one or both tags. Because neither group disclosed a tagged polymerase/beta and/or gamma tagged dNTP interactive sequencing strategy such as a tagged polymerase/beta and/or gamma tagged dNTP FRET strategy, these two references, along with the belief in the art at the time, teach firmly away from the tagged polymerase/beta and/or gamma tagged dNTP interactive strategies of this invention.

To an ordinary artisan, the Korlach et al. specification is not enabling as to any beta or gamma dNTP except those defined in the cited articles in the Korlach et al. specification, where disclosed ANS is directly bonded to the gamma-phosphate of a dNTP. Thus, Korlach et al. did not disclose how to make beta or gamma dNTPs, did not disclose any beta or gamma labeled structure, and did not disclose any method for making beta or gamma dNTPs, except the structure and methods disclosed in the cited journal articles.

In further support of the contention that Korlach et al. did not possess an invention relating to a sequencing strategy involving detecting interactions between incorporation of beta or gamma labeled nucleotides and labeled polymerase, Applicants point to the fact that Korlach et al. refer to **nucleotide analogs** in their originally filed claims. Applicants will further elaborated on this matter after listing the claims of the 17 May 2000 Korlach et al. application:

> 1. A method of sequencing a target nucleic acid molecule having a plurality of nucleotide bases comprising:
>
> providing a complex of a nucleic acid polymerizing enzyme and the target nucleic acid molecule oriented with respect to each other in a position suitable to add a nucleotide analog at an active site complementary to the target nucleic acid;
>
> providing a plurality of types of nucleotide analogs proximate to the active site, wherein each type of nucleotide analog is complementary to a different nucleotide in the target nucleic acid sequence;

polymerizing a nucleotide analog at an active site, wherein the nucleotide analog being added is complementary to the nucleotide of the target nucleic acid, leaving the added nucleotide analog ready for subsequent addition of nucleotide analogs;

identifying the nucleotide analog added at the active site as a result of said polymerizing; and

repeating said providing a plurality of types of nucleotide analogs, said polymerizing, and said identifying so that the sequence of the target nucleic acid is determined.

2. A method according to claim 1, wherein the nucleic acid polymerizing enzyme is selected from the group consisting of a DNA polymerase, an RNA polymerase, reverse transcriptase, and mixtures thereof.

3. A method according to claim 1, wherein the nucleic acid polymerizing enzyme is a thermostable polymerase.

4. A method according to claim 1, wherein the nucleic acid polymerizing enzyme is a thermodegradable polymerase.

5. A method according to claim 1, wherein the target nucleic acid molecule is selected from the group consisting of double-stranded DNA, single-stranded DNA, single stranded DNA hairpins, DNA/RNA hybrids, RNA with a recognition site for binding of the polymerase, and RNA hairpins.

6. A method according to claim 1, wherein the nucleic acid polymerizing enzyme is bound to the target nucleic acid molecule complex at an origin of replication, a nick or gap in a double-stranded target nucleic acid, a secondary structure in a single-stranded target nucleic acid, a binding site created by an accessory protein, or a primed single stranded nucleic acid.

7. A method according to claim 1, wherein the nucleic acid polymerizing enzyme is provided with one or more accessory proteins to modify its activity.

8. A method according to claim 7, wherein the accessory protein is selected from the group consisting of a single-stranded binding protein, a primase, and helicase.

9. A method according to claim 1, wherein the nucleic acid polymerizing enzyme is processive.

10. A method according to claim 1, wherein the nucleic acid polymerizing enzyme is non-processive.

11. A method according to claim 1, wherein the nucleotide analogs are selected from the group consisting of a ribonucleotide, a deoxyribonucleotide, a modified ribonucleotide, a modified deoxyribonucleotide, a peptide nucleotide, a modified peptide nucleotide, and a modified phosphate-sugar backbone nucleotide.

12. A method according to claim 1, further comprising:

hybridizing an oligonucleotide primer to the target nucleic acid molecule prior to or during said providing a plurality of nucleotide analogs.

13. A method according to claim 12, wherein the oligonucleotide primer comprises nucleotides selected from the group consisting of ribonucleotides, deoxyribonucleotides, modified ribonucleotides, modified deoxyribonucleotides, peptide nucleic acids, modified peptide nucleic acids, and modified phosphate-sugar backbone nucleotides.

14. A method according to claim 1, wherein the nucleotide analogs are provided with a label.

15. A method according to claim 14, wherein the label is selected from the group consisting of chromophores, fluorescent moieties, enzymes, antigens, heavy metals, magnetic probes, dyes, phosphorescent groups, radioactive materials, chemiluminescent moieties, scattering or fluorescent nanoparticles, Raman signal generating moieties, and electrochemical detection moieties.

16. A method according to claim 14, wherein the label is attached to the nucleotide analog at its base, sugar moiety, alpha phosphate, beta phosphate, or gamma phosphate.

17. A method according to claim 14, wherein the label is attached to the nucleotide analog with a linker.

18. A method according to claim 14, wherein the label is attached to the nucleotide analog without a linker.

19. A method according to claim 14 further comprising:
removing the label from the nucleotide analog during or after said identifying and before said polymerizing many further nucleotide analogs at
the active site.

20. A method according to claim 19, wherein said removing is carried out by bleaching the label.

21. A method according to claim 20, wherein said bleaching is carried out by photobleaching with radiation which is adjusted to induce and control label removal.

22. A method according to claim 19, wherein said removing is carried out by cleaving the label from the nucleotide analog.

23. A method according to claim 22, wherein beta- or gamma-labeled nucleotide analogs are enzymatically cleaved.

24. A method according to claim 14, wherein each of the plurality of types of nucleotide analogs have different labels which are distinguished from one another during said identifying.

25. A method according to claim 14, wherein three or less of the plurality of types of nucleotide analogs have a different label.

26. A method according to claim 14, wherein the different types of nucleotide analogs have the same label but are distinguished by different properties due to the presence of base fluorophores, quenched fluorophores, or fluorogenic nucleotide analogs.

27. A method according to claim 1, wherein the nucleic acid polymerizing enzyme carries a label and said identifying is carried out by detecting interaction between the label and the nucleotide analog.

28. A method according to claim 27, wherein the label is a fluorescence resonance energy transfer donor or acceptor.

29. A method according to claim 1, wherein said identifying is carried out by non-optical procedures.

30. A method according to claim 1, wherein said identifying is carried out by optical procedures selected from the group consisting of far-field microspectroscopy, near-field microspectroscopy, evanescent wave or wave guided illumination, nanostructure enhancement, and combinations thereof.

31. A method according to claim 1, wherein said identifying is carried out by utilizing single and/or multiphoton excitation, fluorescence resonance energy transfer, or photoconversion.

32. A method according to claim 1, wherein said identifying is achieved by spectral wavelength discrimination, measurement and separation of fluorescence lifetimes, fluorophore identification and/or background suppression.

33. A method according to claim 32, wherein fluorophore identification and/or background suppression utilizes fast switching between excitation modes and illumination sources, and combinations thereof.

34. A method according to claim 1, wherein said providing a complex comprises:

positioning either (1) an oligonucleotide primer or (2) the target nucleic acid molecule on a support;

hybridizing either (1) the target nucleic acid molecule to the positioned oligonucleotide primer or (2) an oligonucleotide primer to the positioned target nucleic acid molecule, to form a primed target nucleic acid molecule complex;
and

providing the nucleic acid polymerizing enzyme on the primed target nucleic acid molecule complex in a position suitable to move along the target nucleic acid molecule and extend the oligonucleotide primer at an active site.

35. A method according to claim 34, wherein said hybridizing is carried out by additionally binding the end of the target nucleic acid molecule opposite to that bound to the oligonucleotide primer to a second oligonucleotide primer positioned on the support.

36. A method according to claim 34, wherein the support and either the oligonucleotide primer or the target nucleic acid molecule are bound reversibly or irreversibly with corresponding components of a covalent or non-covalent binding pair selected from the group consisting of an antigen-antibody binding pair, a streptavidin-biotin binding pair, photoactivated coupling molecules, and a pair of complementary nucleic acids.

37. A method according to claim 34, where the oligonucleotide primer is positioned on the support and the target nucleic acid molecule is hybridized to the positioned oligonucleotide primer.

38. A method according to claim 34, wherein the target nucleic acid molecule is positioned on the support and the oligonucleotide primer is hybridized to the positioned target nucleic acid molecule.

39. A method according to claim 1, wherein said providing a complex comprises:

positioning, on a support, a double stranded nucleic acid molecule comprising the target nucleic acid and having a recognition site proximate the active site, and

providing the nucleic acid polymerizing enzyme on the target nucleic acid molecule in a position suitable to move along the target nucleic acid molecule.

40. A method according to claim 1, wherein said providing a complex comprises:

positioning a nucleic acid polymerizing enzyme on a support in a position suitable for the target nucleic acid complex to move relative to the nucleic acid polymerizing enzyme.

41. A method according to claim 40, wherein the support and the nucleic acid polymerizing enzyme are bound reversibly or irreversibly with corresponding components of a covalent or non-covalent binding pair selected from the group consisting of an antigen-antibody binding pair, a streptavidin-biotin binding pair, photoactivated coupling molecules, and a pair of complementary nucleic acids.

42. A method according to claim 1, wherein the nucleic acid polymerizing enzyme or the target nucleic acid is positioned on an adjustable support.

43. A method according to claim 1, wherein the nucleic acid polymerizing enzyme or the target nucleic acid is positioned in a gel with pores.

44. A method according to claim 1, wherein the target nucleic acid and the nucleic acid polymerizing enzyme are positioned on a solid support proximate to each other.

45. A method according to claim 1, wherein said identifying is carried out by reducing background noise resulting from free nucleotide analogs.

46. A method according to claim 45, wherein said identifying comprises:
directing activating radiation to a region substantially corresponding to the active site and detecting the nucleotide analog polymerized at the active site.

47. A method according to claim 45, wherein said identifying distinguishes nucleotide analogs polymerized at the active site from free nucleotide analogs.

48. A method according to claim 45, wherein said identifying is carried out in a confined region proximate to the active site.

49. A method according to claim 48, wherein said identifying is carried out in a nanostructure.

50. A method according to claim 49, wherein the nanostructure is a punctuate, acicular, or resonant nanostructure which enhances said detecting.

51. A method according to claim 48, wherein nucleotide analogs that are not polymerized at the active site move rapidly through a microstructure to and from the confined region.

52. A method according to claim 51, wherein the microstructure comprises:
a plurality of channels to direct different nucleotide analogs to the confined region and
a discharge channel to permit materials to be removed from the confined region, and the nanostructure comprises:
a housing defining the confined region and constructed to facilitate said identifying.

53. A method according to claim 45, wherein said identifying is carried out by electromagnetic field enhancement with electromagnetic radiation being enhanced proximate to an object with a small radius of curvature adjacent to the active site.

54. A method according to claim 45, wherein said identifying is carried out by near-field illumination of cavities in which the primed target nucleic acid molecule is positioned.

55. A method according to claim 45, wherein said identifying is carried out with optical fibers proximate to the complex.

56. A method according to claim 45, wherein said identifying and said reducing background is carried out by time gated delay of photon detection.

57. A method according to claim 1, wherein said method is carried out by sequencing different target nucleic acid molecules at a plurality of different locations on an array.

58. A method according to claim 1, wherein said method is carried out by simultaneously or sequentially sequencing the same target nucleic acid and combining output from such sequencing.

59. An apparatus suitable for sequencing a target nucleic acid molecule comprising:
a support;
a nucleic acid polymerizing enzyme or oligonucleotide primer suitable to bind to a target nucleic acid molecule, wherein said nucleic acid polymerizing enzyme or said oligonucleotide primer is positioned on said support;
and
a microstructure defining a confined region containing said support and said nucleic acid polymerizing enzyme or said oligonucleotide primer and configured to permit labeled nucleotide analogs that are not positioned on the support to move rapidly through the confined region.

60. An apparatus according to claim 59, wherein the microstructure comprises:

a plurality of channels to direct different types of nucleotide analogs to the confined region and

a discharge channel to permit materials to be removed from the confined region and a nanostructure constructed to facilitate identification of nucleotide analogs positioned on the support.

61. An apparatus suitable for sequencing a target nucleic acid molecule comprising:

a support;

a nucleic acid polymerizing enzyme or oligonucleotide primer suitable to hybridize to a target nucleic acid molecule, wherein said nucleic acid polymerizing enzyme or said oligonucleotide primer is positioned on said support;

a housing defining a confined region containing said support and said nucleic acid polymerizing enzyme or said oligonucleotide primer and constructed to facilitate identification of labeled nucleotide analogs positioned on the support; and

optical waveguides proximate to the confined region to focus activating radiation on the confined region and to collect radiation from the confined region.

First, Applicants note that nowhere in these claims does Korlach et al. couple beta or gamma labeled dNTPs with an interactive label strategy such as a strategy based on interaction between beta or gamma labeled dNTPs and a labeled polymerase like a FRET strategy.

Moreover, Korlach et al. speak of nucleotide analogs in their original claims. Original claim 1 of the May 17, 2000 Korlach et al. filing reads as follows:

1. A method of sequencing a target nucleic acid molecule having a plurality of nucleotide bases comprising:

providing a complex of a nucleic acid polymerizing enzyme and the target nucleic acid molecule oriented with respect to each other in a position suitable to add a nucleotide analog at an active site complementary to the target nucleic acid;

providing a plurality of types of nucleotide analogs proximate to the active site, wherein each type of nucleotide analog is complementary to a different nucleotide in the target nucleic acid sequence;

polymerizing a nucleotide analog at an active site, ***wherein the nucleotide analog being added is complementary to the nucleotide of the target nucleic acid, leaving the added nucleotide analog ready for subsequent addition of nucleotide analogs***;

identifying the nucleotide analog added at the active site as a result of said polymerizing; and

repeating said providing a plurality of types of nucleotide analogs, said polymerizing and said identifying so that the sequence of the target nuclei acid is determined.

Korlach et al. use the term "nucleotide analog" in the above claim, especially in the text set in italics. Close examination of the use the term "nucleotide analog" in the non-italics text and the italics text follows.

The clause "***wherein the nucleotide analog being added is complementary to the nucleotide of the target nucleic acid***" would be understood by one skilled in the art as referring to the first use of the term in the claim and relates to the nucleotide analog being covalently attached to extend the

DNA strand using Watson-Crick base pairing rules. The clause "*leaving the added nucleotide analog ready for subsequent addition of nucleotide analogs*" would be interpreted by one skilled in the art as meaning that the nucleotide analog (modified nucleotide) that was incorporated in the DNA strand is in a state that allows for the additional incorporation of nucleotide analogs. However, the use of the term "nucleotide analog" in this clause implies to one skilled in the art that a **nucleotide analog** is present at the 3' end of the nascent DNA strand - not a natural nucleotide as results from incorporation of beta or gamma modified nucleotides. Merrian-Webster's Medical Dictionary defines an analog as "a chemical compound that is structurally similar to another but differs slightly in composition (as in the replacement of one atom by an atom of a different element or in the presence of a particular functional group)." While the Encarta World English Dictionary defines an analog as "a chemical with a similar structure to another but differing slightly in composition." An ordinary artisan would understand this claim to mean that the incorporated nucleotide was not a natural nucleotide, but was modified in some way. This same term, "nucleotide analog", is also found throughout the Korlach et al. specification.

Thus, when the cited Korlach et al. reference was filed on May 17, 2000, Korlach et al. were not in possession of an invention involving labeled polymerase and beta or gamma labeled nucleotides in a tag interactive strategy, because the incorporation of beta or gamma labeled nucleotides does not give rise to an incorporated nucleotide analog, but rather a natural nucleotide – the resulting DNA contains only natural nucleotides.

Although Korlach et al. does disclose beta and gamma labeled dNTPs, **they did not disclose** the use of beta or gamma labeled dNTPs in an interactive tag strategy such as FRET. Nothing in their 1999 or 2000 documents supports a sequencing strategy using a donor labeled polymerase and beta or gamma labeled dNTPs. Moreover, because Applicants can antedate the 17 May 2000 Korlach et al. specification and original claims, the only Korlach et al. application that included beta or gamma modified dNTPs, Applicants are free of Korlach et al. in any sequencing strategy involving non-persistently labeled nucleotides.

Claims 71-77

As to claims 71-77, Korlach et al. discloses specifically beta or gamma phosphate labeled nucleotides. On the other hand, Applicants disclosed the use of **monomers** and defined monomers as "any compound that can be incorporated into a growing molecular chain by a given polymerase." Applicants also clearly disclosed labeling the beta and gamma phosphate directly or through a linker. Applicants' monomer definition and the use of terminal phosphate does relate to the gamma

phosphate as the terminal phosphate, but it also relates to monomers that may have more than three phosphate groups. In fact, some recently synthesized monomers include additional phosphates so that the terminal phosphate may be the 4th, 5th, *etc.* phosphate. Korlach et al is silent on the issue of labeling beyond the base, sugar or alphas, beta or gamma phosphate.

Again, Korlach et al. does not disclose any interactive tag sequencing strategy, where a non-persistently labeled nucleotide label interacts with a labeled polymerase. Thus, regardless of the type of non-persistently labeled nucleotides (labeled directly at the beta or gamma phosphate or via a linker the may include additional phosphate groups), Korlach et al. simply does not disclose such tag interactive strategies. Like Schneider et al., who do not even disclose non-persistently labeled nucleotides, Korlach et al. is limited to interactive strategies involving persistently labeled nucleotides and labeled polymerases.

Because Korlach et al does not disclose the invention of claims 50-55, 57-62, 64-69, and 71-77, Korlach et al cannot anticipate these claims, and Applicants, therefore, respectfully request withdrawal of this section 102 rejection. Moreover, it is also clear Korlach et al. do not even suggest such a strategy and can render claims 50-55, 57-62, 64-69, and 71-77 obvious.

Applicants would like to point out to the Examiner that in several of the claims including claim 17, Applicants added as one of the fluorescent properties duration along with frequency and intensity. Support for this addition can be found at least at paragraph number 0088 (this paragraph number relates to the USPTO published application text.

Claim Rejections - 35 USC § 103

Claims 79-87 and 89-98 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Korlach et al. (US 2006/0078937 Al) in light of Wisniewski et al. and Gardner et al. as applied to claims 10, 13-18, 50-55, 57-62, 64-69, and 71-77 above, and further in view of Schneider et al. (US 6,982,146 B1). This rejection is necessitated by amendment.

The Examiner contends as follows:

> Korlach et al. in light of Wisniewski et al. and Gardner et al. describe the limitations of 10, 13-18, 50-55, 57-62, 64-69, and 71-77, as stated in the 35 USC 102 rejection above. Korlach et al. describe the limitations in dependent claims 82-87 and 92-98, as documented in the 35 USC 102 rejection above. Korlach et al. do not describe where the site comprises a naturally occurring cysteine site in the polymerizing agent selected so that the site is less than or equal to about 25Å, 15Å, or 10Å from a tag on each incorporating monomer regions and are not sites having

structural/functional importance to proper functioning of the polymerizing agent and is covalently bonded to the cysteine through its SH group (claims 79-81 and 89-91).

Schneider et al. describe attachment of fluorophores to a polymerase (col. 19, line 57 to col. 20, line 35). Schneider et al. describe using thiol-reactive probes to a generate fluorescentlylabeled polymerase where the thiol (SH) groups that are present in the cysteine residues react with the fluors to yield chemically stable thioesters (col. 20, second paragraph), noting that fluorescently-labelled polymerases have high fluorescent yield and retain critical features of the polymerase thus preserving the function of the polymerase (col. 20, lines 26-32) as well as the limited distance of 10A between a donor fluorophore on a polymerase and a target acceptor fluorophore on a nucleotide without collateral stimulation of other acceptor fluorophores (col. 9, fifth paragraph), as stated in instant claims 79-81 and 89-91.

Korlach et at. state a need to provide a method for sequencing nucleic acid molecules that requires only polymerase activity, without the use of blocking substituents, resulting in greater simplicity, easier miniaturizability, and compatibility to parallel processing of a single-step technique (paragraph 0013). It would have been obvious to the person of ordinary skill in the art at the time the invention was made to modify the method of Korlach et al. by attaching the molecular tag to a cysteine site as taught by Schneider et at. where the motivation would have been to meet today's demand for rapid, high-throughput sequencing and accurately sequence nucleic acids at the molecular scale at high speed and long reading lengths, without requiring the labor intensive use of electrophoresis or complex liquid pumping systems, via fluorescentlylabeled polymerases that retain the ability to synthesize a complementary strand and have a high fluorescent yield, as stated by Schneider et al. (col. 3, fifth paragraph and col. 20, fifth paragraph) and Korlach et al. (paragraphs 0004-0005 and 0010).

Thus, Korlach et al. in light of Wisniewski et al. and Gardner et al. and in view of Schneider et al. make obvious claims 10, 13-18, 50-55, 57-62, 64-69, 71-77, 79-87, and 89-98.

Applicants incorporate by reference all of the arguments regarding Korlach et al. here. While Schneider et al. do disclose labeling at cysteine groups, they do not disclose where the label should be placed:

> Alternatively, thiol-reactive probes can be used to generate a fluorescently-labeled polymerase. In proteins, thiol groups are present in cysteine residues. Reaction of fluors with thiols usually proceeds rapidly at or below room temperature (RT) in the physiological pH range (pH 6.5 8.0) to yield chemically stable thioesters. Examples of thiol-reactive probes that can be used include, but are not limited to: fluorescein, BODIPY, cumarin, rhodamine, Texas Red and their derivatives.

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Like Korlach et al., Schneider et al. do not disclose, teach or suggest where such labels should be placed. Clearly, one cannot rely exclusively on native cysteines because some polymerases do not include native cysteines, *e.g., Taq* has no cysteines. (One of Applicants' reasons for using *Taq* as a model polymerase). Other enzymes include cysteines that are not accessible. Other enzymes

include numerous available cysteines, but without a more detailed labeling plan – a plan that is unique to the Applicants specification – labeling a polymerase with multiple cysteines would result in non-specifically labeled enzymes and would decrease the consistency of the signal produced during nucleotide incorporation. Although Schneider et al. state that limiting the distance will help limit transfer to the desired target, they do not provide guidance for a suggested distance. There is no disclosure, teaching or even suggestion as to how one goes about selecting a suitable, unique site for polymerase labeling except for fusion proteins.

As to the teaching on col. 9 of Schneider et al., this is a general teaching of the FRET distance. It only speaks to that fact that the acceptable distance for observing FRET fluorescence is between 10 and 100Å. However, there is no disclosure, teaching or suggestion as to how one goes about tagging a polymerizing agent using cysteine to satisfy this requirement. Clearly, cysteine tagging will not work for *Taq* as *Taq* has no cysteines. So whatever Schneider et al. are teaching has nothing to do with a generalized labeling process without more specific teaching of mutagenesis of sites in polymerases with a cysteine codon at a site that does not interfere with polymerase activity for polymerases without cysteine. Schneider et al. simply do not include a strategy for teaching an ordinary artisan how to use cysteines to prepare appropriately tagged polymerases.

As to the teaching of col. 19 line 57 to col. 20, line 35, although Schneider et al. teach that these techniques, including thiol-labeling are well known in the art, what is not well known in the art is where to and how to position the thiol-label. Schneider et al. do not disclose, teach or suggest a strategy for positioning the thiol-label. Clearly, one can attach a label to any cysteine that is present and accessible on a polymerase, but Schneider et al. **DO NOT** disclose, teach or suggest how to select the site at which to introduce a cysteine if one is not present, or how to label one if several cysteine sites exist such that the donor label is positioned to support intense FRET signals for incorporating tagged dNTPs. For example, there are sites on polymerases that are not suited for labeling regardless of whether a cysteine is present. Thus, thiol-labeling of this site would either not give rise to a functioning sequencing composition or would not be an effective donor for a FRET sequencing strategy.

Moreover, tagging of a polymerase is not a random affair. If a tag is to be attached so that a distance between the polymerase tag and a tag on an incorporated nucleotide is within a given distance such as between 100Å and 10Å, the polymerase tag must be positioned with great care. In other embodiments, Applicants set the distance as less than or equal to (within) 50Å, 25Å, 15Å or 10Å. Applicants' method provides specific and clear guidance for positioning donor tags so that

intense acceptor fluorescence, via FRET, are detected and analyzed to identify incorporation events. The present application is the only application that provides a methodology for selecting polymerase labeling sites, adding a cysteine if needed and labeling even in circumstances where more than one cysteine is present.

Even the combination of Korlach et al and Schneider et al. do not to disclose, teach or suggest the limitations of claims 79-87 and 89-98, because the combination gives no guidance as to label attachment site selection. The combination clearly recognizes that FRET has a distance limitation, but the combination does not provide any teaching on the relative distance between the label on the polymerase and the label on incorporating nucleotides, save for fusion proteins, where a portion of the polymerase is fluorescently active. Without disclosure at as to proper placement of cysteine in the polymerase, the combination does not enable, as it would require undue experimentation to determine where to and how to place the a label on a polymerase. The combination of Korlach et al and Schneider et al. give absolutely no guidance on how or where, save the mention of thiol-labeling.

Additionally, as previously stated, the 3' to 5' exonuclease activity limitation is not present in the combination making claims 79-87 even more non-obvious. Moreover, the combination does not disclose, teach or suggest monomers labeled through the beta and/or gamma phosphate via a linker, regardless of the nature of the linker, which could include additional phosphate groups or any other group.

Applicants also point out that it seems rather odd to indicate that claims 56, 63, 70, and 78 represent patentable subject matter, while claims 88 and 99, equivalents of claim 56, 63, 70, and 78 are not. Moreover, Applicants do not understand how the results of the above strategy produce allowable claims (specific labeling sites on *Taq*), while the strategy that dictates the proper way to select cysteine sites as set forth in independent claims 79 and 89, is not. The former claims are totally dictated by the latter claims.

Applicants also point out that Schneider et al. teach using polymerases that include '3 to 5' exonuclease activity:

> Polymerase: The enzyme which catalyzes the elongation of the primer strand, in the 5' to 3' direction along the nucleic acid template to be sequenced. Examples of polymerases which may be used in the method disclosed herein include, but are not limited to: the E. coli DNA polymerase I, specifically the Klenow fragment which has 3' to 5' exonuclease activity, Taq polymerase, reverse transcriptase, E. coli RNA polymerase, and wheat germ RNA polymerase 11.
> ...

the E. coli DNA polymerase I, **specifically the Klenow fragment which has 3' to 5' exonuclease activity**

Schneider et al.

Thus, Schneider et al. did not disclose, teach or suggest that the polymerase lack 3' to 5' exonuclease activity, which is consistent with the teaching of Korlach et al. as it relates to polymerases. In fact, these experts, Schneider et al., specifically teach away from polymerases lacking 3' to 5' exonuclease activity as is clear in the highlighted portion of the above quote.

The present invention specifically sets forth the requirement for labeling and for polymerizing agents lacking 3' to 5' exonuclease activity or a polymerizing agent that lacks the ability to remove a previously incorporated monomer, and these limitations are patentably distinct from the combination. Applicants respectfully request withdrawal of this section 103 rejection.

Applicants Summarize the Rejection

The Examiner contends as follows:

> Applicants submit that they formally antedate Korlach et al. as it pertains to the use of beta phosphate, gamma phosphate, and/or terminal phosphate labeled dNTPs in single molecule sequencing, and request withdrawal of the 35 USC 102 rejection as it relates to claims 50-55, 57-78, and 89-99. It is noted that claims 57-78 and 89-99 are new claims and therefore were not previously rejected. It is noted that the 35 USC 102 rejection of claims 10, 13-18, 50-55, 57-62, 64-69, and 71-77 is necessitated by amendment for the reasons given above. Applicants submit that the present invention was made before May 17, 2000, and the Applicants' attorney can attest via documentary evidence that the present invention was invented prior to May 17, 2000, which is the date of filing of the Korlach 09/572530 application. These statements are found unpersuasive as they are insufficient allegations that lack any factual support. It is noted that filing a declaration under 37 CFR § 1.131 would be considered improper, because it is a potential interference with the claims of the prior art reference. Applicants are advised to file the appropriate papers under 37 CFR § 41.202 or § 41.203, as appropriate.
>
> Applicants argue that Korlach et al.'s provisional application does not disclose beta and/or gamma phosphate labeled dNTPs. This statement is moot as the filing date of Korlach et al.'s 09/572530 application (May 17, 2000) is sufficient for making the 35 USC 102(e) rejection above. Applicants argue that Korlach et al. do not disclose terminal phosphate labeled nucleotides. This statement is found unpersuasive as Korlach et al. disclose a monomer tag covalently bonded to the α, β, or γ phosphate group of each dNTP (claim 66 and paragraphs 0064-0067).
>
> Applicants reiterate arguments regarding antedating the prior art reference which were found unpersuasive as stated above. Applicants request withdrawal of the 35 USC 102(e) rejection of claims 50-55, 57-78, and 89-99. It is noted that claims 57-78 and 89-99 are new claims and therefore were not in the previous

rejection. It is noted that the 35 USC 102 rejection of claims 10, 13-18, 50-55, 57-62, 64-69, and 71-77 is necessitated by amendment for the reasons given above.

Applicants summarize Korlach et al.'s (May 17, 2000) application (now US 7,056,661) and argue that this application does not support using beta and/or gamma phosphate labeled dNTPs in the context of FRET detection of incorporating events. This statement is found unpersuasive as US 7,056,661 makes reference to using beta and gamma phosphate labeled dNTPs (col. 14, last paragraph to col. 15, first paragraph) and FRET (col. 26).

Applicants argue that US 6,982,146 (Schneider et al.) does not anticipate or render obvious claims 50-55, 57-78, and 89-99 because of failure to disclose labeling dNTP at the β and/or γ phosphate or terminal phosphate. This statement is found moot as Korlach et al. disclose these limitations (claim 66 and paragraphs 0064-0067).

Applicants reiterate that they have formally antedated Korlach et al.'s 09/572530 application as it pertains to beta, gamma, and terminal phosphate labeled dNTPs. This statement is again found unpersuasive for reasons given above. Applicants submit that Korlach et al.'s provisional application does not recite beta, gamma, and terminal phosphate labeled dNTPs. This statement is again found moot for reasons already given above. Applicants argue that the provisional does not render claims 50-55, 57-78, and 89-99 obvious and summarize the provisional application. These statements are considered moot as an obvious rejection for this provisional application was not made.

Applicants note amendments made to claims 10 and 13-18. Applicants argue that Korlach et al. do not disclose polymerases lacking 3' to 5' exonuclease activity. This statement is found unpersuasive as this activity is an inherent characteristic for Sequenase, Taq DNA polymerase I, and reverse transcriptase, including HIV-1 reverse transcriptase (seeWisniewski et al. (page 28175, col. 2, first paragraph) and Gardner et al. (page 606, col. 1, last paragraph)). Applicants argue that Schneider et al. do not disclose polymerases lacking 3' to 5' exonuclease activity. This statement is found moot as this activity is inherent for Sequenase, Taq DNA polymerase I, and reverse transcriptase, including HIV-1 reverse transcriptase, as stated above.

Applicants argue that Korlach et al. nor Schneider et al. render claims 79-88 obvious as there is no teaching in these references regarding polymerases lacking 3' to 5' exonuclease activity, the proper placement of the tag on the polymerizing agent that should be less than or equal to 25Å from the incorporating monomers, or that the site be located on a site that is not important to the proper functioning of the polymerizing agent. These statements are unpersuasive as the characteristic of polymerases lacking 3' to 5' exonuclease activity is an inherent characteristic for Taq DNA polymerase I, Sequenase, and reverse transcriptase (including HIV-1 reverse transcriptase) and the other limitations listed above are described by Schneider et al. (col. 19-20).

Applicants submit that they have fully responded to the Examiner's Final Office Action. It is noted that the previous Office Action was a non-final Office Action.

Applicants' arguments are deemed unpersuasive for the reasons given above.

Applicants have noted these comments from the Examiner and consider them moot based on the arguments and claim amendments set forth above.

Applicants note the Examiner's statement relative to terminal phosphate, which in dNTPs is the gamma phosphate. However, this is not the case in all monomers, where the terminal phosphate or linker can be a polyphosphate group.

Applicants also note the Examiner's statement "[t]his statement is found unpersuasive as US 7,056,661 makes reference to using beta and gamma phosphate labeled dNTPs (col. 14, last paragraph to col. 15, first paragraph) and FRET (col. 26)," but these two statements are not connected in the application. As stated above, **nowhere in the Korlach et al. specification and original claims, do they disclose, teach or even suggest using beta or gamma labeled nucleotide in an interactive label strategy such as a FRET strategy** (see paragraph [0104] of Korlach et al. and above discussions).

Applicants again point out that although some of the polymerizing enzymes in Korlach et al. and Schneider et al. lack 3' to 5' exonuclease activity, Korlach et al. used T7 in many of their examples, an enzyme that has 3' to 5' exonuclease activity and Schneider et al. preferred enzymes with 3' to 5' exonuclease activity. These facts argue squarely against the Examiner's position on this issue.

Applicants, therefore, urge allowance of the claims of this invention over the cited prior art.

Allowable Subject Matter

Claims 56, 63, 70, and 78 are objected to as being dependent upon a rejected base claim, but would be **allowable** if rewritten in independent form including all of the limitations of the base claim and any intervening claims. Claims 56, 63, 70, and 78 recite specific amino acid positions of SEQ ID NO: 11 from Taq DNA polymerase I for attachment to a tag which are not disclosed in the prior art.

Applicants acknowledge the allowability of these claims, **noting that claims 88 and 99, equivalents of claim 56, 63, 70, and 78 are not**. Applicants, however, urge the Examiner to also pass into allowability the more general disclosure of how to determine where to tag polymerases, a strategy not disclosed by any of the cited references.

The Commissioner is authorized to charge or credit deposit account 501518 for any fees due or any overpayments, respectively.

If the Examiner requires additional information, then Applicants request that the Examiner contact their Attorney, Robert W. Strozier, at 713-977-7000.

Date: **1 February 2007**

Respectfully submitted,

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